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Springboard

Data Science Career Track

Capstone Final Report

Alzheimer's, Genetics, and the Microbiome-Gut-Brain Axis: How Naturopathic Medicine Affects Alzheimer's

The Problem

The United States Secretary of the Department of Health has declared for many years that the public health crisis of Alzheimer's is an ongoing federal problem that US citizens face. The gravity of this disease is very serious and can be fatal. Today, "nearly 7 million Americans live with Alzheimer's" [1] , and "the cost of care yearly is 360 billion USD" [2]. "10% of adults aged 45 and older report subject cognitive decline" [3], which is the earliest warning sign of Alzheimer's disease. "By 2050, nearly 13 million Americans could be living with Alzheimer's [4], where costs will reach nearly $1 trillion" [5].

Figure. 1 below is taken from the "2024 Alzheimer's Disease Facts and Figures" report from the Alzheimer's Association® [6].

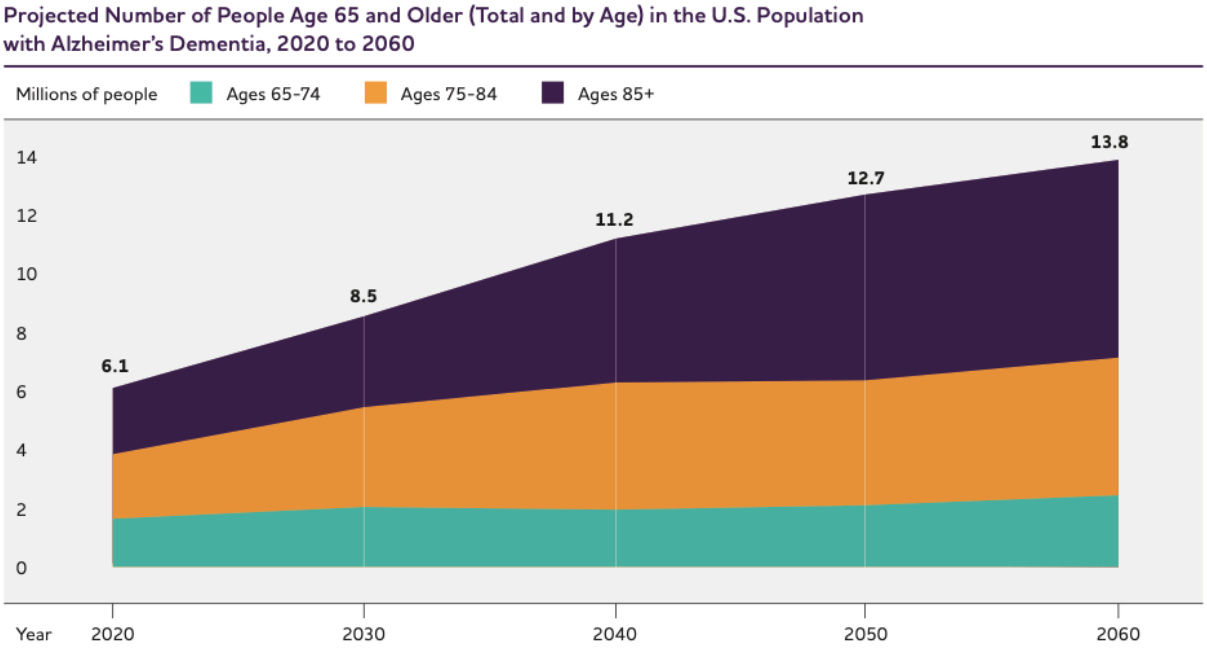

Figure. 1 shows the Alzheimer's Disease projection by year 2060.

What is Naturopathic Medicine?

Conventional medicine is a system designed for a brief consultation with a medical doctor, typically lasting 15 minutes. The goal is to diagnose as quickly as possible and to treat ailments or diseases with prescribed pharmaceuticals. However, this approach often fails to address the root causes of systemic issues affecting a patient's whole body.

Conventional medicine struggles to address the public health crises facing Americans. While it is "highly effective in addressing acute and life-threatening conditions" [7] through surgery and necessary medications, it often falls short as a definitive model for combating public health crises such as Alzheimer's. This limitation contributes to problems such as the opioid crisis and increased rates of ADHD. Although pharmaceuticals are essential in many cases, they are not a comprehensive solution for addressing the functional and holistic health of a patient's entire body, which influences a patient's disease status.

This is where Naturopathic Medicine comes in. This medicine is a science-based paradigm that aims to treat the whole body by identifying and addressing the root causes of disease. It seeks to improve all health markers of a patient's bodily systems through protocols involving nutraceuticals, diet, nutrition, health tests, lifestyle therapies, and natural treatments, allowing a patient's body to heal itself completely or to its fullest extent. Naturopathic Medicine "was founded in the United States in 1901 by Benedict Lust" [8], taken from a movement that was happening at the turn of the century in Europe.

Figure. 2 on the next page depicts the fundamental principles of Naturopathic Medicine.

Figure. 2 shows the 6 Principles of Naturopathic Medicine [105].

Therapeutic Order and Course of Action in Naturopathic Medicine

1. **First Layer:** *Establish the Foundation for Optimal Health:* A Naturopathic Doctor (ND) educates patients on the basics of living healthy, laying the groundwork for long-term health.

2. **Second Layer:** *Stimulate the Body's Natural Healing Power:* Initially, an ND prescribes supplements or nutraceuticals to address inflammatory or oxidative "bottlenecks." This helps alleviate high levels of inflammation contributing to an ailment [9].

3. **Third Layer:** *Support & Restore the Body's Weakened Systems:* After the baseline education, supplements, and nutraceuticals to handle the major sources of inflammation that contribute to a patient's ailments, protocols and programs of supplements, nutraceuticals, natural therapies, diet, nutrition, and lifestyle changes seek to naturally restore a body's weakened systems completely, or to their fullest possible extent [10]. The following are some biomarker tests that assess the health of bodily systems, along with an example of how they may be weakened. They are labeled from first to last in layers like the pyramid in Figure. 3 as shown below, to also indicate what is more fundamental to overall health.

Bodily Health Systems: Their Sequential Importance and Interconnection

1. **First Layer (Bottom of the Pyramid):** *Gastrointestinal Tract:* A stool test assesses the gut microbiome and intestinal health markers to identify toxic burdens and inflammation from bacteria, pathogens, infections, and overall digestion [11] [12] [13].

2. **Second Layer:** *Liver and Detox Tract:* A hair follicle and mold test assesses heavy metal toxicity and mold levels in a person to evaluate how well a patient is managing oxidative stress and detoxification, which contribute to aging and ailments [14] [15] [16].

3. **Third Layer:** *Hormonal System:* A saliva test that evaluates reproductive hormones' ratios and quantities, which are crucial for inflammation and overall wellbeing. Example: A woman who has chronically low levels of estrogen, which affects her mood and inflammation levels [17] [18] [19].

4. **Fourth Layer:** *Adrenal System:* A day-long saliva test measures cortisol levels throughout the day to assess adrenal fatigue, better known as "burnout" [20] [21] [22].

5. **Fifth Layer:** *Neurotransmitter Test:* A saliva panel evaluates the ratios of neurotransmitters, essential for mental wellbeing [23] [24] [25].

6. **Sixth Layer (Top of the Pyramid):** *Genetics Test:* A saliva test assesses which genes may contain mutations, potentially indicating "dirty genes" needing to be cleaned up. Improvement of the first layer health system improves markers in all subsequent layers. Improving all health system layers before the Genetics Layer consequentially improves the status of the genetics layer [26] [27] [28].

Therapeutic Order and Course of Action in Naturopathic Medicine (continued)

4. **Fourth Layer:** *Targeted Natural Prescriptions:* After weakened bodily systems are completely restored or restored to their fullest possible extent, if there is still a deficiency within the health systems of a patient or other kinds of deficiencies and bottlenecks, then whatever the nutraceuticals from the second layer cannot address is addressed by the addition of targeted natural prescriptions [29].

5. **Fifth Layer:** *Replacement Therapy:* After targeted natural prescriptions are issued, there might still be deficiencies within a patient. For example, testosterone-boosting supplements such as Fadogia Agrestis and Tongkat Ali are potent natural interventions, but a male patient still suffering from low

testosterone will benefit from testosterone replacement therapy [30] [31]. This is a recommended course of action to address this highly inflammatory imbalance.

6. **Sixth Layer:** *Drug Therapy:* At this stage, if there is still a need to address imbalances within a patient, prescription pharmaceuticals are recommended.

7. **Final Layer:** *Surgery:* The final stage is invasive surgery to address any structural or biological imbalances that require this level of attention.

Important Note: Since we are a whole body and all of our bodily systems are interconnected as a whole, a heavier inflammatory load in the first layer is a heavier load on all of the following sequential layers. A heavier inflammatory and oxidative load on the fourth layer is a heavier load for the fifth through last layers, etc.

"Conventional medicine typically only addresses replacement therapy, drug therapy, and surgery while ignoring the first four layers of fundamental health."

Figure. 3 in the next page depicts the Therapeutic order of Naturopathic Medicine.

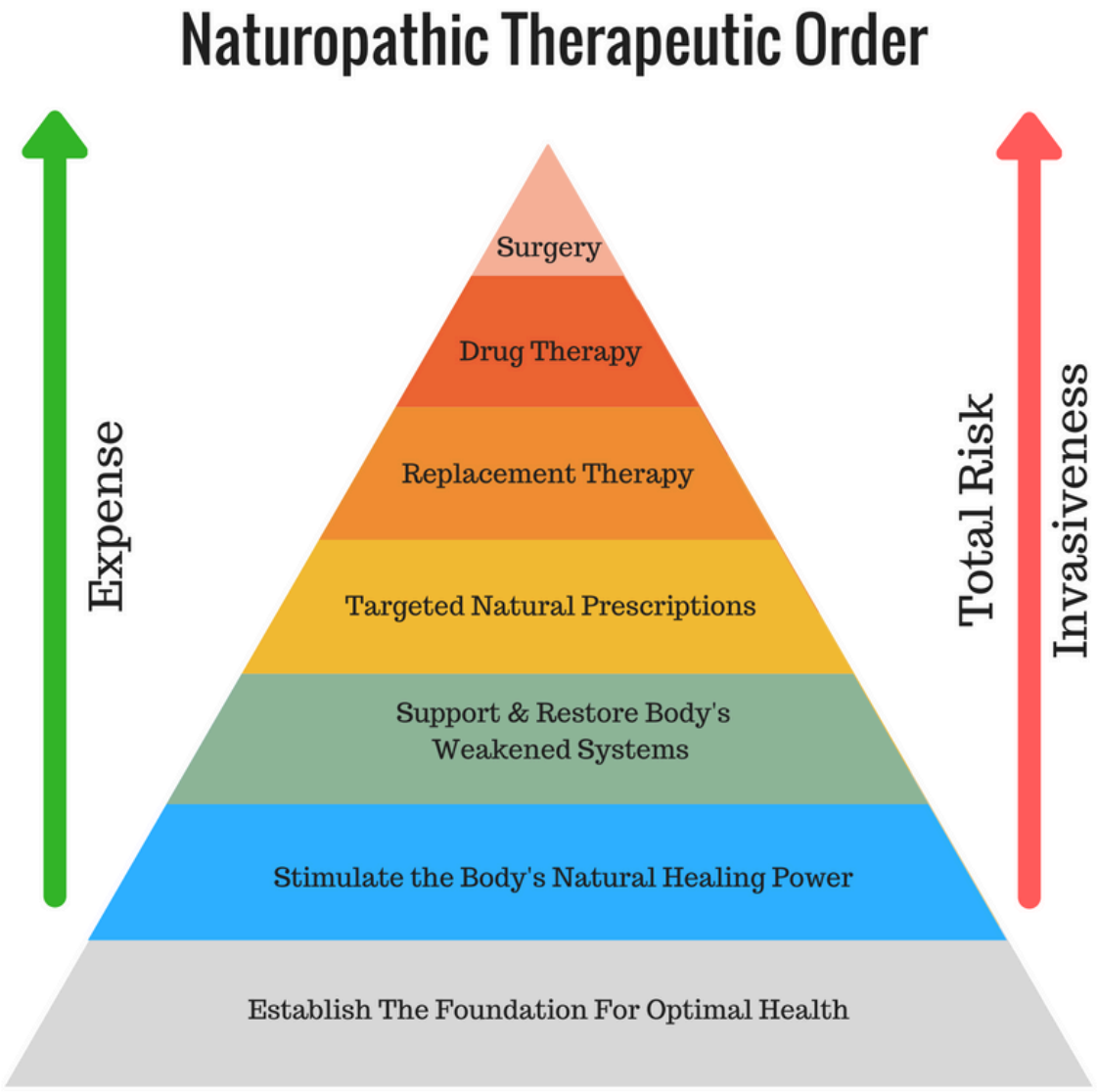

Figure. 3 shows the Seven Layers of Therapeutic Order in Naturopathic Medicine [106].

An Example of a Naturopathic Course of Action

For instance, a Naturopathic Doctor (ND) might order a stool test to assess biomarkers of gastrointestinal health. Based on the results, the ND would prescribe nutraceuticals or herbal supplements as part of a baseline protocol to address imbalances in the patient's GI tract. If a patient has experienced rheumatoid arthritis from a young age, with pain stemming from systemic inflammation caused by all bodily systems, the nutraceuticals, and herbal supplements might help correct imbalances such as low stomach acid (hypochlorhydria) caused by a common bacterial infection in the GI tract, H. pylori. The ND might also recommend additional supplements for cleansing and repairing the GI tract to eliminate dysbiosis at later dates [32].

What is Traditional Chinese Medicine?

Traditional Chinese Medicine (TCM) is an ancient practice that spans thousands of years, that has changed little over the centuries. TCM is based on the fundamental concepts of "Yin-Yang" and "Qi". See Figure. 4 below for a depiction of this fundamental concept of TCM. "A vital force of life, called Qi, surges through the body. Any imbalance in Qi can cause disease and illness. This imbalance is most commonly thought to be caused by an alteration in the opposite and complementary forces that make up the Qi... called Yin and Yang" [33].

"The first writings about TCM date back to 200 BCE. Herbal medicine, acupuncture, including theory, practice, diagnosis, and treatment, were recorded in classical Chinese texts and [was] refined over many centuries" [34].

Disease is thought to be alterations in the normal flow of Qi such that Yin and Yang are imbalanced. TCM agrees that an unbalanced Yin and Yang typically has three major causes: external or environmental factors, internal thoughts and emotions, and lifestyle factors. Like Naturopathic Medicine, TCM stimulates the body's own natural restoration and healing processes.

Practices used in TCM include:

1. Herbal medicine
2. Nutrition
3. Acupuncture and acupressure
4. Moxibustion: Burning a herb near the skin
5. Chinese massage: Called Tui Na
6. Exercise: Such as Tai Chi and Qi Gong which combine movement with meditation

TCM like Naturopathic Medicine also believes that our bodily systems are not individual networks, but rather "complex networks" [35]. "According to TCM, Qi flows through organ systems, the kidneys, heart, spleen, liver, lungs, gallbladder, small intestine, and large intestine, by way of meridians"[36] also known as energy pathways.

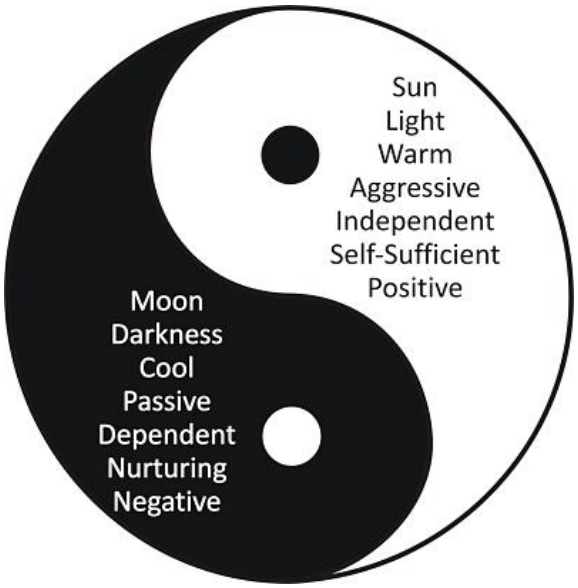

Figure. 4 shows the Fundamental Concept in Traditional Chinese Medicine [107].

Naturopathic Medicine & Traditional Chinese Medicine Compared

"The term "naturopathy" originates from "natura" (Latin root for birth) and "pathos" (the Greek root for suffering) to suggest "natural healing". Naturopaths claim the ancient Greek "Father of Medicine", Hippocrates, as the first advocate of Naturopathic Medicine, before the term existed"[37].

Traditional Chinese Medicine stems from "The Yellow Emperor's Inner Canon (Huangdi Neijing), the oldest received work of Chinese medical theory, [which] was compiled during the Han dynasty around the first century BCE" [38]. "It was also one of the first books in which the cosmological doctrines of Yinyang...were brought to a mature synthesis" [39].

Naturopathic Medicine like Traditional Chinese Medicine where both founded circa 200 BC. Both ancient practices aim to restore a body's natural ability to heal itself through natural purposes because both practices believe that disease is caused by imbalances within the person. Hippocrates who is the "Father of Medicine" postulated that these imbalances primarily stemmed from one's own temperament. The following section aims to describe the vital importance of these temperaments, and how they relate to modern medical theory as a body of my own original work from a lifetime of study. The following paradigm is my life's work which maps temperament and modern medicine together.

Hippocrates' Humoral Theory, Naturopathic Medicine, Modern Medicine, and Archetypes

In medieval and ancient times, all Medical Doctors were instructed in the premise of "Humoral Theory" which was founded by Hippocrates [40]. Humoral Theory states that all people may be categorized by four different types of temperaments, which are marked by excesses in a specific kind of humor [41]. Humor comes from the word human, humor and human from the same root word Humus in Latin which means "earthly". To be human is to have excess or deficiencies of certain humors. "Hippocrates suggested that humors are the vital bodily fluids: blood, phlegm, yellow bile, and black bile [42]. Hippocrates postulated that an extreme excess or deficiency of any of the humors (bodily fluid) in a person can be a sign of illness"[43]. Figure. 5 below shows a German 16th-century drawing of the 4 temperments. Hippocrates lived from 460 – c. 370 BC.

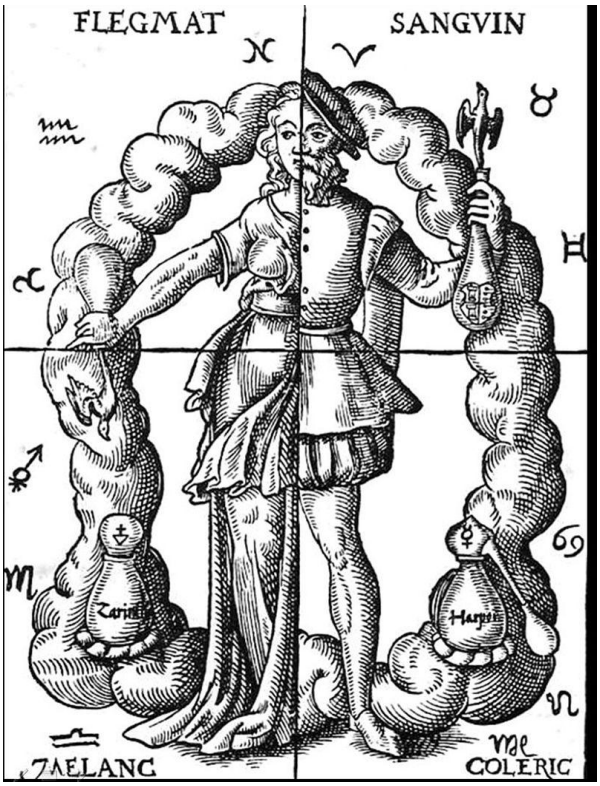

Figure. 5 shows a drawing from the 16th-century of The 4 Temperments in Humoral Theory [108].

The Phlegmatic Temperment

Figure. 6 shows The Phlegmatic Temperment in Humoral Theory [109].

There is much wisdom in making the philosophical claim that excesses and deficiencies, in certain bodily fluids affect your temperament. Take the Phlemagtic temperament. The Phlegmatic is categorized as having an excess bodily fluid of phlegm [44]. Having an excess of phlegm is marked by a later scientific discovery known as "rest and digest" [45]. This is where the parasympathetic system is activated, and all of your bodily energy is focused on resting and digesting food after it is consumed [46]. "Rest and Digest" is opposed to "Fight or Flight" [47] which is marked by feeling a sense of danger or preoccupation where someone feels the need to flee a situation or fight a situation that is usually life-threatening [48]. The "Fight or Flight" state is marked by very

high levels of elevated cortisol [49], the release of adrenaline [50], and catecholamines [51], which is what makes the whole "Fight of Flight" experience a stressful experience.

In "Rest and Digest" the digestive system is activated to break down food, so nutrients may be absorbed [52]. Conditions known as "Burnout" or medically known as "Adrenal Fatigue" are marked by chronically low levels of cortisol, which occurs after someone is constantly pathologically being in a state of "Fight or Flight" [53].

The temperaments go much deeper than just biological processes. Someone who is Phlegmatic, who is constantly in a state of "Rest and Digest", is temperamentally marked as someone who is always calm and never stressed. These are people who are very easygoing. But this goes even deeper still. Because someone is temperamentally "always calm, never stressed, and easy-going", from an Aristotelian-Thomistic perspective on virtues, they can be prone to laziness [54]. Aristotelian-Thomistic virtue ethics states all temperaments have inherent strengths and weaknesses [55].

Someone who is Phlegmatic, who is constantly in a state of "Rest and Digest" is said to have a high concupiscible appetite, which is defined as someone "Who has a great appetite to move towards many pleasurable inherent goods, and move away from arduous evils" [56]. As such, this temperament is naturally very stoic, calm, and peaceful but they may be prone to laziness and be less dutiful from an Aristotelian-Thomistic perspective on virtue ethics that are dispositions in someone's character [57].

The Choleric Temperment

Figure. 7 shows The Choleric Temperment in Humoral Theory [110].

The Phlegmatic temperament is as opposed to the Choleric Temperament, which is marked by a high irascible appetite which is defined as "a great appetite to move towards arduous inherent goods, and away from easy evils" [58]. Hippocrates in Humoral Theory marked the Choleric Temperament as someone who has an excess of the humor "yellow bile" [59]. It was later discovered by modern science that yellow bile is a product that is stored in the gallbladder of a person, that sits just under the liver [60]. Yellow bile is a product of detoxed toxins from stage II detoxification pathways before they are used one more time in the digestive system to help break down fats which are the most calorically dense macronutrient you can eat [61].

The Choleric temperament goes deeper. Since "yellow bile" is the result of detoxed toxins that an individual interfaces with on a daily basis, this temperament is defined as someone who is a "leader", "very hard worker", and "spearhead of work" [62]. Hence, since so much of this temperament aligns with "doing", this temperament

will inevitably absorb more toxins through the skin, and lungs, which inevitably hits our livers to be dealt with in a detoxification process [63].

Someone who is a Choleric, who is constantly working to arduous goods, may be more prone to anger hence the word Choleric which comes from the Latin word for anger "Cholera" [64]. They are typically less tolerant of people and their mistakes from an Aristotelian-Thomistic perspective on virtue ethics within someone's character [65]. They normally deal with more stress than all the other temperaments, so they may be more easily prone to adrenal fatigue or "burnout" [66] [67]. There is much wisdom from Hippocrates' school of thought and his Humoral Theory indeed.

The Melancholic Temperment

Figure. 8 shows The Melancholic Temperment in Humoral Theory [111].

The Melancholic temperament is on a different axis than what the Phelgmatics or Cholerics are on. The Phlegmatic is to what is to "be" or "rest", where you do not have to think, feel, or do anything to "be". You just are the summation of everything that you think, feel, or do. Choleric is to "doing", it is an active principle of human nature.

Melancholics are on the thinking and feeling axis, whereas Melancholics are to "reflection", and "discovering and believing the truth for the sake of truth" [68], the final temperament Sanguine is to "feeling" and " feeling interconnected with everything" [69].

Hippocates stated that Melancholics have an excess of "black bile" which was a liquid that was found in the spleen at the time of Hippocates [70]. "Black Bile was thought to originate in the spleen, meaning that melancholy... [was]...blamed on the spleen. With an overproduction of Black Bile, a person may have appeared cold and dry, sad in disposition, and overall doubtful of the world" [71]. Modern medicine states that the spleen "stores and filters blood and makes white blood cells that protect you from infection" [72] and "the spleen is part of the lymphatic system" [73]. The lymphatic system deals with the waste that is naturally left behind from natural blood circulation within blood plasma [74].

Since fluid from the spleen is a concentration of white blood cells and lymph, modern medicine affirms that excesses in spleen fluid mark someone who biologically produces more waste in their natural blood circulation [75], hence the reflective nature of this temperament, because "doing" things, and their actions that follow, may lead to greater waste that is produced by their blood exertion in "doing things", which is then picked up by the lymphatic system in blood plasma [76], which connects to the spleen [77].

Someone who is a Melancholic, who is constantly thinking to understand truths, may be more prone to melancholy hence the word Melancholic which comes from the Latin word for sadness "Melancholicus". They typically react more slowly and are affected for a long time from negative events, in an Aristotelian-Thomistic perspective on virtue ethics within someone's character [78]. Aristotelian-Thomistic philosophy states that this stems from this temperament of "never wanting to suffer" [79] hence them falling into a character defect known as fear [80]. Fear is defined as "the feeling that an inevitable evil is coming your way, that you cannot stop" [81] which is why this temperament is by nature also very reflective because they do not want to suffer negative things [82]. This can be offset by growing in the virtues of hope, joy, and gratefulness for one's state in life [83].

Melancholics normally deal with higher levels of serotonin than all the other temperaments, which is an inhibitory neurotransmitter, they are inhibitory towards making rash decisions. Modern Medicine states serotonin and dopamine work in a

push-pull fashion, when one goes lower the other tends to go higher [84]. A Sanguine typically has high constant levels of dopamine, and thus lower levels of serotonin. Dopamine is an excitatory feel-good neurotransmitter for "pursuing goals" [85], and serotonin is the inhibitory feel-good neurotransmitter for "feeling content with what you have" [86]. Melancholics are more easily prone to melancholy or "anxiety" because anxiety is marked by not having enough dopamine to "chase rewards" as a precursor to adrenaline that is released in perceived stressful situations in an anxiety disorder [87]. There is much wisdom from Hippocrates' School of Thought and his Humoral Theory, which still relates to Modern Medicine today.

The Sanguine Temperment

Figure. 9 shows The Sanguine Temperment in Humoral Theory [112].

The final temperament of the four temperaments is the Sanguine Temperament. It is on the same axis as the melancholic temperament. Melancholic is to thinking, as Sanguine is to feeling. The Phlegmatic is to "being", and Choleric is to "doing".

Hippocrates stated that Sanguine's have an excess of the blood fluid [88]. Sanguines are defined as "very charismatic people", they are very social, they want to be connected to everything and they want to feel everything, hence the flow the fluid from the heart [89]. Heart for feeling, blood for passion, and whole body interconnectedness.

Philosophically, the Sanguine differs from the Melancholic in that Sanguines do not have to deal with excess waste within the plasma in their natural blood circulation, which the Melancholic's spleen and lymph have to deal with. The Melanchonics have higher rates of waste in their blood circulation by nature, and the Sanguine does not. The Sanguines philosophically deal with less circulating waste in their spleen and plasma, hence why they want to "feel everything" [90] and be "connected to everything in nature" [91], to have a sense of "oneness" [92] that starts in the ventricles of their hearts.

Sanguines are defined from an Aristotelian-Thomistic perspective as "reacting very quickly to negative events, and being undisturbed by them" [93], which is the exact opposite of a Melancholic who "reacts very slowly to negative events, and is affected for a long time by them" [94].

Someone who is a Sanguine who reacts very quickly to negative events, remains undisturbed by them, is very social and very charismatic is from an Aristotelian-Thomistic view "someone who is prone to intemperance" from the dispositions within their character regarding their virtues [95]. Aristotelian-Thomistic philosophy states why this stems from this temperament, it is because by nature they tend to "never want to be apart from pleasure" [96] hence them falling into intemperances such as drug addiction and alcoholism, in which alcoholism is found to have a genetic predisposition within families [97] that are Sanguine. This points to the Modern Medical concept of Epigenetics which states in general that "The summation of all your experiences, good and bad, all influence your genetics, and the genes that are passed down to the next generation up until the very moment of conception of a newborn" [98].

Beyond Just Temperments

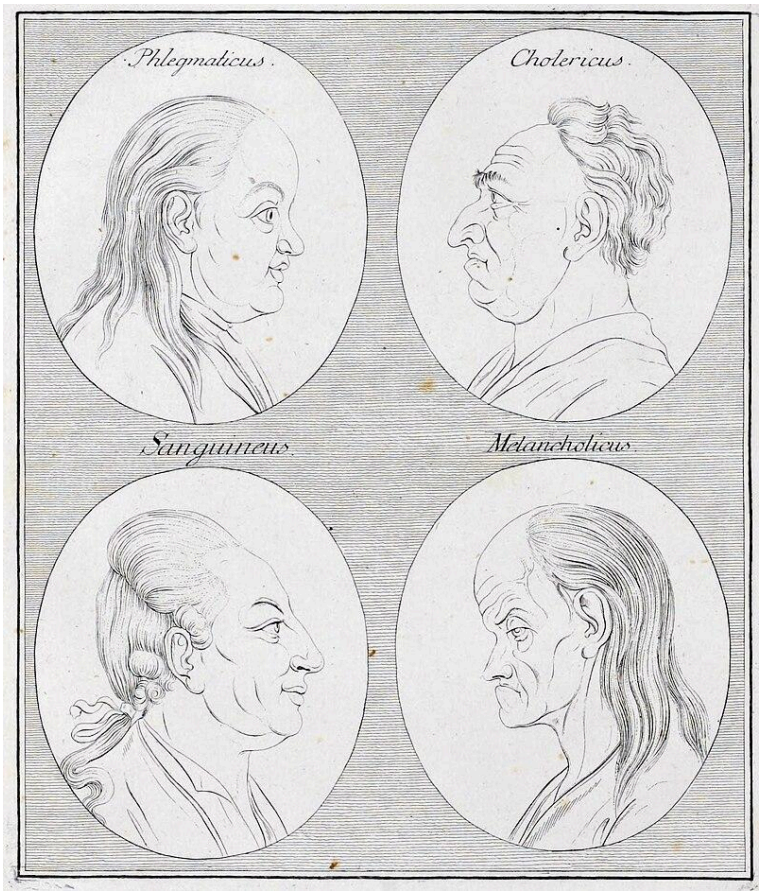

Figure. 10 shows The Faces of the 4 Temperments in Humoral Theory [113].

The paradigm goes much deeper than just temperaments, natural virtue strengths and weaknesses, and biological disease. There is a body of work published by Alexander Lowen who was a student of Wilhelm Reich, a psychologist who was a student of Sigmund Freud, describes in his work that one's temperament and character have "influence in how your anatomy and physiology develops, and in turn, even looks like once adulthood is reached" [99]. That is to say that one's virtues and defects (or character excesses and defects), which symbiotically pair with one's blend of temperments from Hippocrates' Humoral Theory, determine one's own psychology and how one may physically appear in person.

Wilhelm Reich was a direct student of Sigmund Freud [100], Freud pioneered modern Psychology [101]. Reich's "work on character and the idea of muscular armoring contributed to the development of what is now known as... bioenergetic analysis of Reich's student Alexander Lowen" [102] who described formations of human anatomy and physiology as their own kind of "archetype" [103] which is analogous to Hippocrates' Humoral Theory which describes the four temperaments as their own archetypes. Hippocrates's Humoral Theory and Lowen's Bioenergetic Theory are analogous, in that in my own research the temperament archetypes and the "character structure" or "physical appearance" archetypes overlap in their similarities. In other words, each psychological temperament (Humoral Temperament) corresponds to a person's physical appearance in many regards. The body is the mind, and the mind is the body in many respects.

These two sets of archetypes may map to each other, but they are not "hard and fast rules" they are not "completely definitive 100% of the time". It is a metaphysical framework for understanding nature and illnesses. Despite my own life's work in researching and mapping these two paradigms, with the paradigm of modern science, they still describe patterns that can be found within human populations until this day. Patterns are not 100% completely definitive as to what a person's temperament and bioenergetic character structure might actually be, we are usually a blend of two temperaments. These two paradigms only describe archetypes of the human condition, which has been seen since ancient Greek times, and one may postulate that the human condition will not change anytime in the near future. This metaphysical framework still has medical and personal value. Regarding the linking of Hippocrates' Humoral Theory and Lowen's Bioenergetic Analysis, there is much truth to this based on a fundamental concept of biology that states "form follows function" [104] which is from Darwin's Evolution Theory. The nature of nature is all a pseudo-science at its most fundamental concepts, and this is a whole study and discipline that is outside the scope of being further elaborated in this body of work.

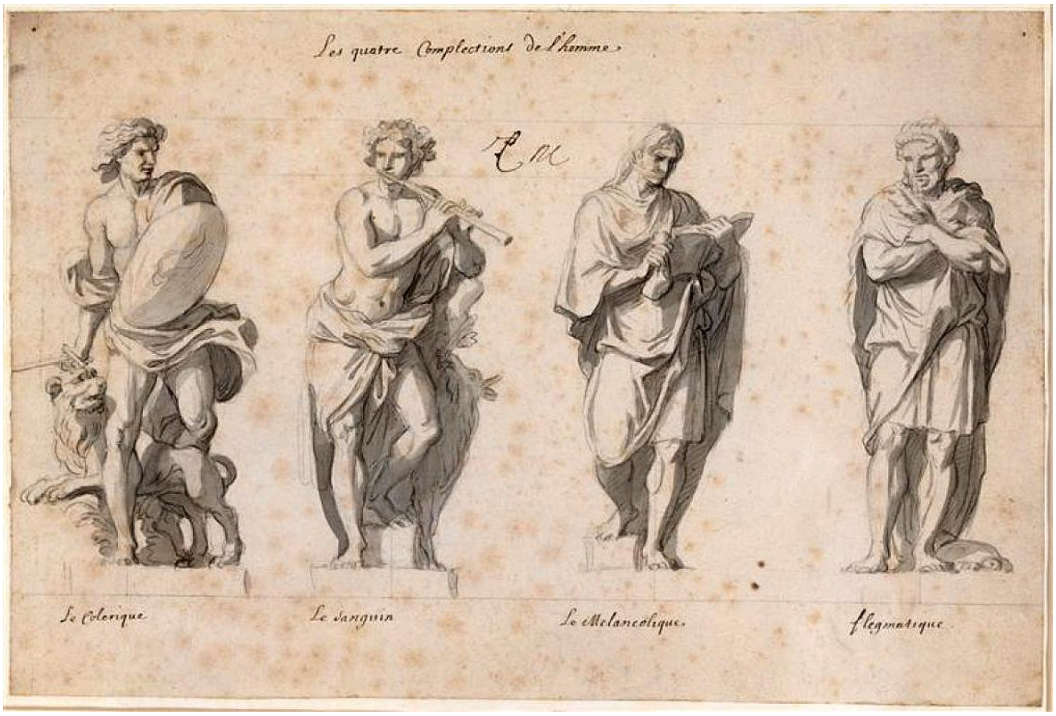

Figure. 11 shows all 4 Temperments in Humoral Theory [114].

The Scope of this Body of Work

The scope of this body of work is limited to the first and final layer (sixth layer) of the "Bodily Health Systems: Their Sequential Importance and Interconnection" section as seen above. This project aims to draw inferences from four datasets which are research studies, that measure biomarkers in genetics, gut microbiota, and biomarkers as seen in the pathology of Alzheimer's disease in the different stages of progression of this pathological phenomenon. Several studies measure the effects of herbal medicine from Naturopathic Medicine and Traditional Chinese Medicine Botany in these two layers of bodily health systems. The purpose of the analysis of this body of work, is to draw inferences from herbal medicine, its effects on gut microbiota, colonic and overall gene expression, and whether or not it has a positive or negative influence on Alzheimer's pathology for research purposes that aim to answer this body of work's project proposal as seen in the section below.

Problem Statement

Alzheimer's is an ongoing public health crisis that Americans face where yearly costs are in the Billions and is expected to grow even more by 2050. It is clear that conventional medicine is insufficient in preventing this public health crisis because it is a systemic 15-minute consultation where the goal is to prescribe a pharmaceutical that only addresses the symptoms of an ailment and not the root causes of an ailment. We have seen earlier in this paper that Naturopathic Medicine addresses every level of what actually contributes to a patient's health in a scientific way.

As such, there are many supplement companies that sell herbal supplements and there are research organizations that could benefit from insights that declare whether or not a herbal supplement is useful in preventing diseases such as Alzheimer's disease. Therefore, the goal of this research paper is to identify how Naturopathic Medicine and Traditional Chinese Medicine herbs, such as Danggui Shaoyao San and Rhodiola Rosea, affect the gut microbiome and thus colonic and overall gene expression, to reduce or prevent the progression of Alzheimer's disease and its pathologies? Purely for research and marketing purposes of companies that would hypothetically market a herbal supplement as "anti-Alzheimer's" or "anti-neurodegenerative".

Brief Preface

In total, there were four datasets that were examined in this project that looked at Alzheimer's, the effects of herbal supplements, and genetics. The goal was to make connections between the datasets, to draw conclusions about how herbal supplements affect genetic biomarkers, inflammatory and oxidative stress biomarkers, and how those biomarkers affect overall Alzheimer's pathology. Let's take a look at the background of each dataset starting with Dataset 1.

Dataset 1

This dataset is taken from a study that aims to see the effects of a Traditional Chinese Medicine Herb known as Danggui Shaoyao San, on modulating and even preventing Alzheimer's Disease.

Danggui Shaoyao San has been used for centuries in Traditional Chinese Medicine, for a variety of purposes, and is composed of 6 herbs. Angelica sinensis (Oliv.) Diels (Umbelliferae), Paeonia lactiflora Pall. (Paeoniaceae), Ligusticum chuanxiong Hort. (Umbelliferae), Poria cocos (Schw.) Wolf (Polyporaceae), Atractylodes macrocephala Koidz. (Compositae), and Alisma orientalis (Sam.) Juzep. (Alismataceae) [125].

Evidence-based Health Benefits of Danggui Shaoyao San:

- 1.) Cognitive Function and Dementia: Has neuroprotective effects, which reduces the risk of dementia [126].
- 2.) Digestive Health: Promotes healthy digestion and stomach function [129].
- 3.) Anti-inflammatory and Antioxidant Effects: Reduces inflammation and oxidative stress in the body [127].
- 4.) Stress and Anxiety Relief: Paeonia lactiflora, may help reduce stress and anxiety, promoting mental relaxation [128].

This study was performed on 30 Thirty Sprague-Dawley (SD) rats, that were separated into three main groups. Two rat groups were injected via bilateral intracerebroventricular injections of streptozotocin (STZ). STZ has toxic effects on insulin-producing cells, which induces insulin resistance for the rats. With induced insulin resistance, and thus impaired brain-insulin sensitivity, Alzheimer's is induced. Alzheimer's is induced in two groups, and there is one control group [130].

"Bloodstream metabolites were characterized, gut microbiota profiled through 16S rDNA sequencing, and cortical metabolomics analyzed. Hippocampal proinflammatory cytokines (IL-1β, IL-6, TNF-α) were quantified using RT-qPCR, and oxidative stress markers (SOD, CAT, GSH-PX, MDA) in brain tissues were measured with biochemical assays" [131].

"The top 45 most abundant species in the gut microbiota were determined across these three groups. Subsequently, Spearman correlation analysis and a heatmap were utilized to assess the correlation between gut microbiota composition and metabolite levels"[132].

Test Groups for Dataset 1 (columns):

- 1.) DSS: A rat group that receives Danggui Shaoyao San (DSS), and is induced with Alzheimer's.
- 2.) M: A rat group that receives zero Danggui Shaoyao San (DSS), and is induced with Alzheimer's.
- 3.) Con: A control rat group that receives zero Danggui Shaoyao San (DSS), and is not induced with Alzheimer's.
- 4.) Description: Biomarkers

Conclusion

Thousands of markers look at bloodstream metabolites, microbiota metabolites, inflammatory cytokine markers, and antioxidative enzyme markers, to draw inferences from how the gut-brain axis affects oxidative stress and neuroinflammation, both of which have been shown to cause Alzheimer's disease. The depth of informative biomarkers from this dataset is quite vast.

Dataset 1 Key Terms

Alzheimer's disease: "Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is characterized by cognitive decline and the presence of two core pathologies, amyloid β plaques and neurofibrillary tangles. Over the last decade, the presence of a sustained immune response in the brain has emerged as a third core pathology in AD" [115].

Inflammation: "Inflammation is a biological response of the immune system that can be triggered by a variety of factors, including pathogens, damaged cells, and toxic compounds. These factors may induce acute and/or chronic inflammatory responses in the heart, pancreas, liver, kidney, lung, brain, intestinal tract, and reproductive system, potentially leading to tissue damage or disease" [116].

> *Sustained overactive inflammation is causal to Alzheimer's, a sustained immune response is indicative of overactive inflammation [117] [118].*

Oxidative Stress: The phenomena of Alzheimer's "are mainly initiated and enhanced by oxidative stress, a process referring to an imbalance between antioxidants and oxidants in favor of oxidants. This imbalance can occur as a result of increased free

radicals or a decrease in antioxidant defense, free radicals being a species that contains one or more unpaired electrons in its outer shell" [119].

> *An imbalanced oxidative stress response is causal to Alzheimer's, such as having too much systemic oxidative stress [120].*

The Gut Microbiome & Inflammation: "The gut microbiota encompasses a diverse community of bacteria that carry out various functions influencing the overall health of the host. These comprise nutrient metabolism, immune system regulation, and natural defense against infection. The presence of certain bacteria is associated with inflammatory molecules that may bring about inflammation in various body tissues. Inflammation underlies many chronic multisystem conditions including obesity, atherosclerosis, type 2 diabetes mellitus, and inflammatory bowel disease. Inflammation may be triggered by structural components of the bacteria which can result in a cascade of inflammatory pathways involving interleukins and other cytokines. Similarly, by-products of metabolic processes in bacteria, including some short-chain fatty acids, can play a role in inhibiting inflammatory processes" [121].

> *A well balanced good gut microbiome is anti-inflammatory, which prevents diseases such as Alzheimer's [122].*

The Gut Microbiome & Oxidative Stress: "Commensal (good) bacteria often exhibit anti-oxidative properties and suppress inflammatory reactions. Pathogenic microbiota induce inflammation and shift the ... balance toward a pro-oxidative status" [123].

> *Having more good gut bacteria is not only anti-inflammatory, it is anti-oxidative, having more "bad" gut bacteria is pro-oxidative [124].*

Data Wrangling Dataset 1

This large dataset from Universities in China was released in 2024. In the description column, there are 4108 biomarkers, ie different categorical biological markers that are measured in this dataset. There were no duplicate rows and there were no missing values so overall this dataset was fairly clean from the start.

Exploratory Data Analysis Dataset 1

The exploratory data analysis of this dataset seeks to answer the following two questions that relate to the progression of Alzheimer's pathology. 1.) *What are the least induced oxidative stress biomarkers in 'DSS Group' vs. 'M Group'? (SOD, CAT, GSH-PX, MDA) [124]. 2.) Preform a "Metabolites" (ie biomarkers) analysis in 'DSS Group' vs. 'M Group', and what does this suggest? [124].*

My approach was to individually filter for known oxidative stress biomarkers that were in the dataset, then I calculated the mean and the standard deviation of all of the oxidative stress biomarkers for the control group, the group that got DSS, and the group that received no DSS.

All of the oxidative stress biomarker averages, and std deviations for the DSS test group are lower than the M group that did not receive DSS, although both groups are induced with Alzheimer's...interesting. This suggests that DSS reduces and prevents Alzheimer's pathology.

	mean_DSS	std_DSS	mean_M	std_M
1	3826.833333	4733.886457	5489.978	8955.680670
2	5796.226667	5804.802297	8678.71	8993.934839

Figure. 12 shows all the means and standard deviations of the DSS group and the M group, taken from the Jupyternotebook of this paper.

Then I formulated the null hypothesis that there is no difference in the DSS group, the M group, and the control group's biomarker data. As it turns out the null hypothesis is rejected that there is no difference in the DSS group, the M group, and the Control group's biomarker data. The DSS group has markedly different biomarker measurements than the other two groups. Lower averages than the M group but higher averages than the control group. This is because the DSS group and the M group both are induced with AD, but their biomarkers differ certainly as seen in the graph in Fig. 13 below.

I then ran permutations of a p-value test to see if the null hypothesis is true (that there is no statistical difference between the DSS group and the M group). As a result of the permuted tests, a p-value was very low which suggests that the null hypothesis be thrown out, thus there are markedly different biomarker measurements between the DSS group and the M group which shows that there are statistically significant differences between both test subject groups as seen in Fig. 14 on the next page.

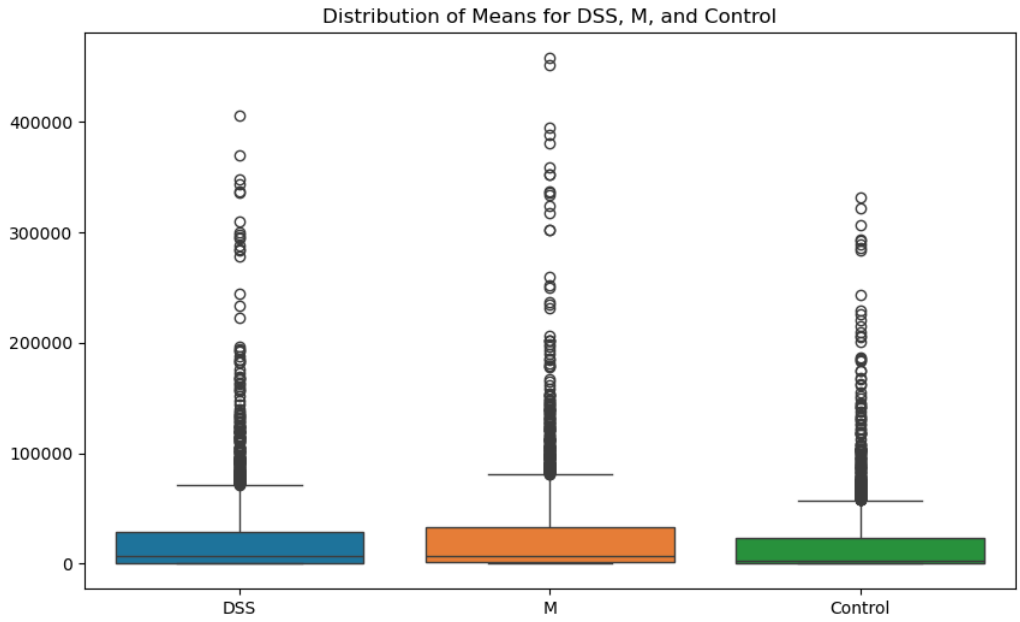

Figure. 13 shows the distribution of the means of the DSS group the M group, and the control group. Taken from the Jupyternotebook of this paper.

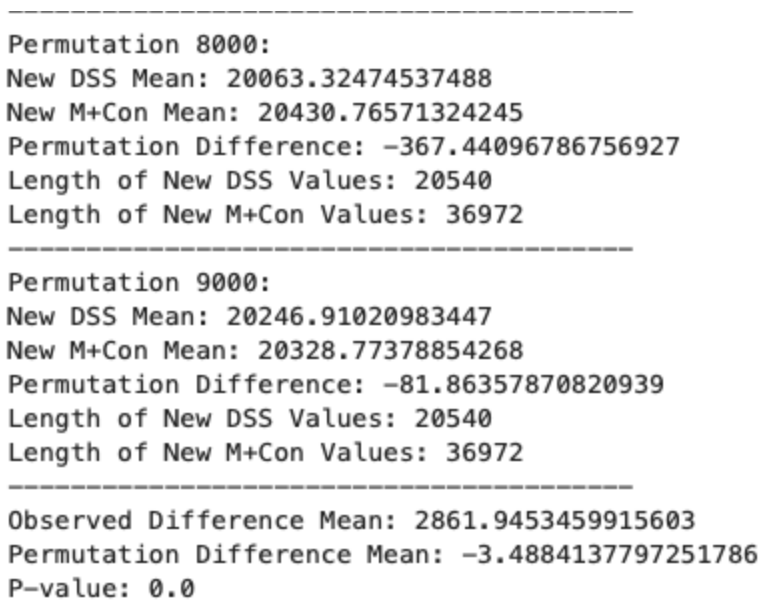

Figure. 14 shows all the p-value permutations of the means of the DSS group the M group + the control group. Taken from the Jupyternotebook of this paper.

Preprocessing and Modeling Dataset 1

It needed to be known how much oxidative stress is associated with test groups (features) within my dataset. That way it can be quantified how prone to Alzheimer's pathology a given feature is associated with whether or not it is the DSS group, the M group, or the control group. For this, I ran a linear regression, a Lasso Regression, and a Ridge Regression. I am predicting a continuous outcome, which is how much the oxidative stress biomarkers are associated with a given feature. I first had to filter for known oxidative stress biomarkers such as NADPH-dependent 2, 4-dienoyl-CoA reductase, and sulfur reductase.

It is useful to cluster the entire dataset's biomarker values to see the clusters of the biomarker values shape up with each other.

Figure. 15 shows the K-means clusters of all the biomarker values from the dataset. Taken from the Jupyternotebook of this paper.

Then I ran a linear regression to see how correlated the DSS group is to the M group, and it is seen that they are positively correlated which means general increases in biomarker values in the DSS group are positively correlated with general increases in biomarker values in the M group.

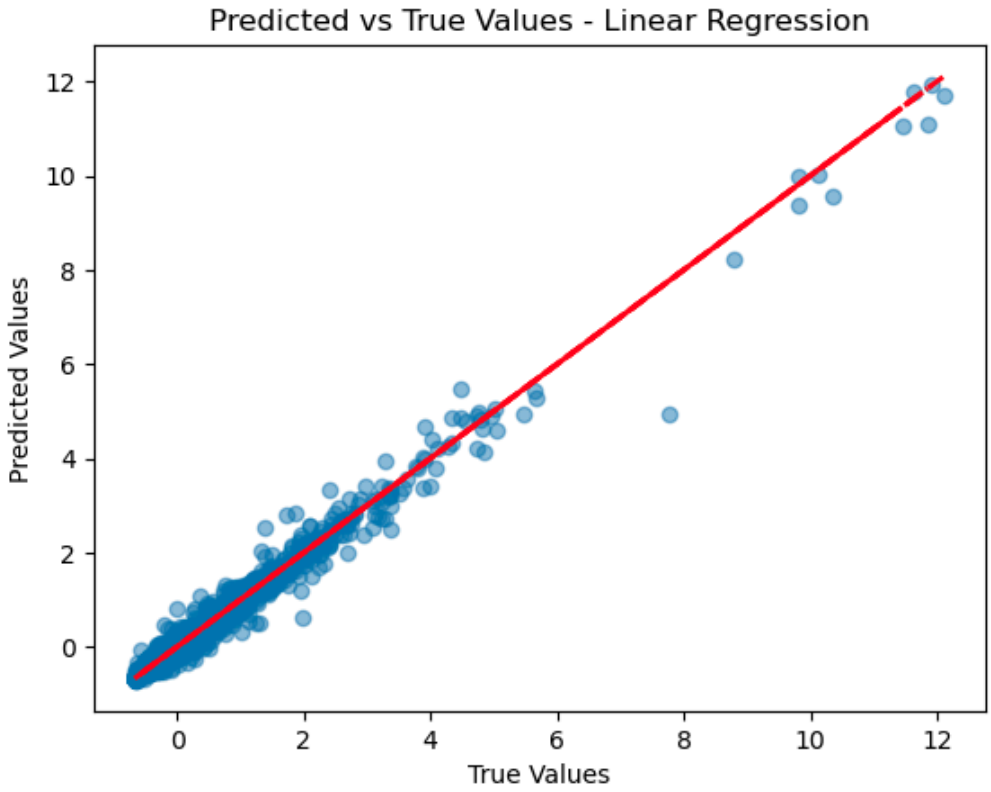

Figure. 16 shows predicting the biomarker values of the DSS group from the M group. Taken from the Jupyternotebook of this paper.

Now I modeled predicting the DSS group's "Oxidative Stress Biomarker values", from the control group's "Oxidative Stress Biomarker Values" to see if there was a correlation there, and there was. The model fits fairly well, and the error is not that bad, it's moderate. Ridge and Lasso did not show dramatic improvement with regularized weights. The results showed to be nearly identical so there was no significant improvement with Ridge or Lasso. This suggests that from the Control group's oxidative stress biomarker values, the DSS group's oxidative stress biomarker values may be somewhat well predicted.

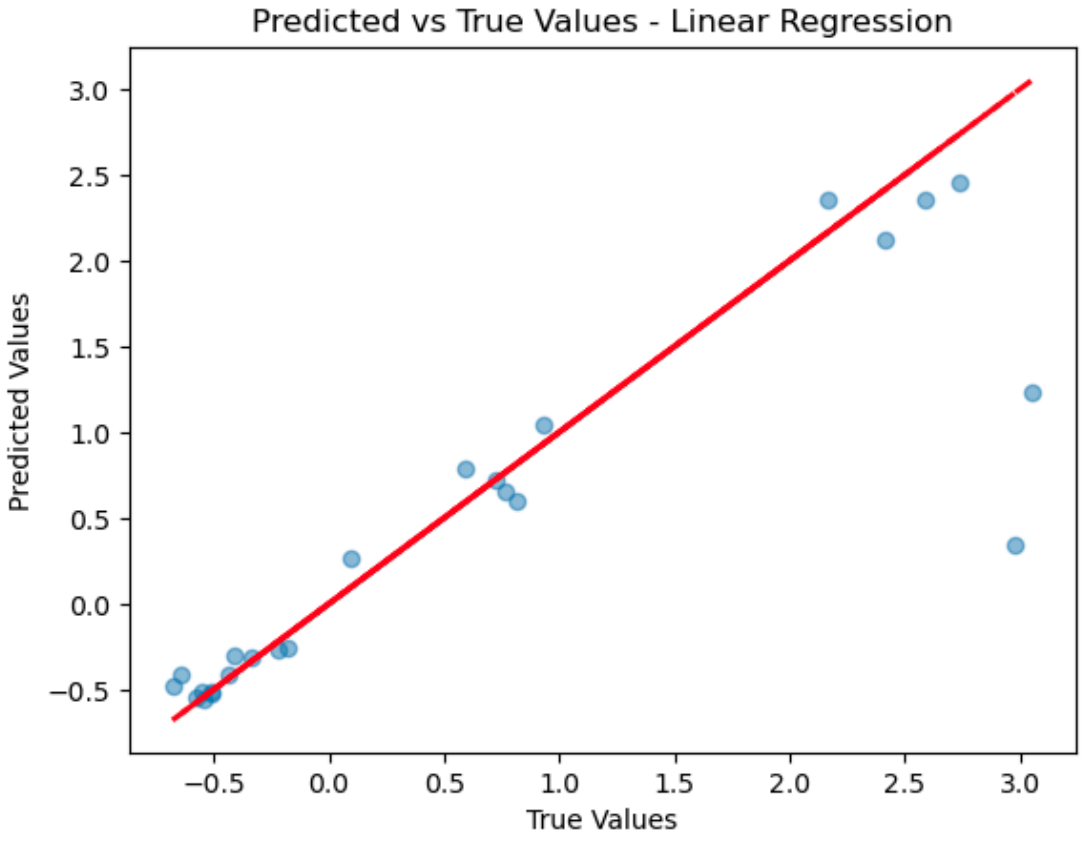

Figure. 17 shows predicting the DSS group's "Oxidative Stress "biomarker values from the control group's "Oxidative Stress" biomarker values. Taken from the Jupyternotebook of this paper.

```
Linear Regression - MSE: 0.450383368980578, R²: 0.7834837141223298
```

Figure. 18 shows the linear regression metrics from Fig 17. Taken from the Jupyter notebook of this paper.

```
Ridge Regression - MSE: 0.450383366427147, R²: 0.7834837150701227
Lasso Regression - MSE: 0.4501992680261535, R²: 0.783428920085136
```

Figure. 19 shows the Ridge and Lasso metrics. Taken from the Jupyter notebook of this paper.

There is also a positive correlation in a linear regression between the M group and the CON group's oxidative stress biomarkers, in predicting the M group's values from the control group's values as seen on the next page in Fig. 21. It is also apparent that the Ridge and Lasso models performed much better in predicting the M group's values from the control group's values. This insight shows higher confidence in predicting higher levels of oxidative stress biomarkers in the M group that was induced with AD with no DSS herbs, compared to the inference that there is less confidence in predicting higher levels of oxidative stress biomarkers in the DSS group and the control group.

This conclusively finds that the models that make predictions on the oxidative stress biomarkers are more confident in predicting higher levels of oxidative stress in the test group that was induced with AD and received no herbal DSS, versus the inference that the models are less confident in predicting a positive correlation between the group that was induced with AD and given DSS herbs, with the control group. In other words, higher levels of oxidative stress are predicted in the M group than the DSS group which points to the idea that Danggui Shaoyao San is efficacious at reducing and preventing oxidative stress, which is a major contributor to Alzheimer's disease pathology.

```
Ridge Regression - MSE: 0.3853119825593684, R²: 0.8103255706541118
Lasso Regression - MSE: 0.38531875048730824, R²: 0.8101734616377823
```

Figure. 20 shows better Ridge and Lasso metrics in predicting the M group's "oxidative stress" biomarkers from the control group, which is better than the DSS group, and the control group's "oxidative stress" biomarkers, showing that regularization of weights proved greater performance with the former. Taken from the Jupyter notebook of this paper.

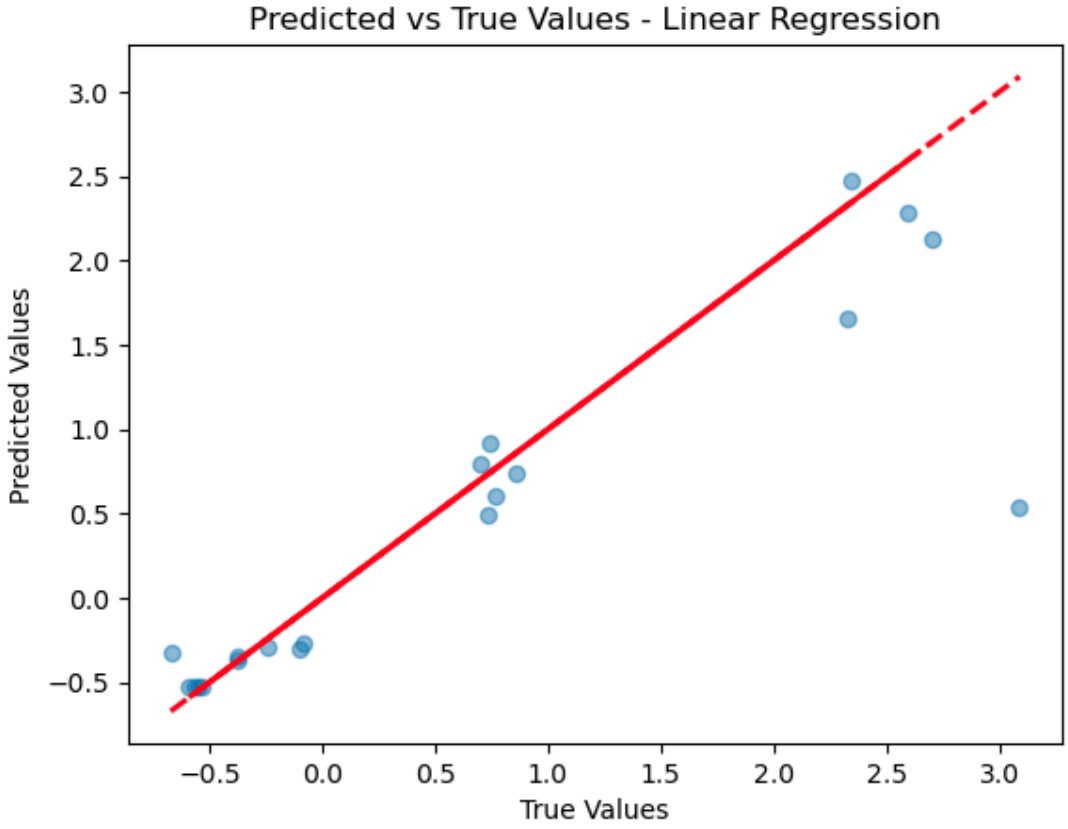

Figure. 21 shows a linear regression in predicting the M group's "oxidative stress" biomarkers from the control group. Taken from the Jupyter notebook of this paper.

Dataset 2

This dataset is a meta-analysis study that analyzes multiple genetic datasets. The study aims to find common differentially expressed genes (DEGs) across multiple microarray experiments (and their resultant datasets), to identify genetic biomarkers, and hub genes for the diagnosis and treatment of Alzheimer's disease (AD).

DEGs from five microarray datasets were identified and intersected to "detect consensus DEGs" [144]. Based on these consensus DEGs a gene Ontology was conducted, and hub genes were identified with the open source platform Cytoscape for visualizing complex networks between these genes.

There were "608 consensus DEGs" and "18 hub genes" [145]. Biomarkers such as "Brain-derived neurotrophic factor (BDNF) and WW domain-containing transcription regulator protein 1 (WWTR1), which are associated with the Braak stage, Aβ 42 levels, and β-secretase activity, were identified as critical genes of AD" [146]. The "study identified 16 hub genes correlated to the neuropathological stage and 35 potential biomarkers for the diagnosis of AD" [147].

Dataset 2 (columns):

- 1.) GSMs: The first column lists the mice specimen.
- 2.) Brain Part Analyzed: The second column lists the brain part analyzed.
- 3.) Gender: Lists the gender of the mice.
- 4.) Braak Stage: Lists the Braak Stage that the mice were at at the time of analysis.
- 5.) All following columns: Biomarkers

Conclusion

The first four columns are categorical descriptions of specimen information, and then there are 19 columns that describe biomarkers that were under analysis that relate to genetics. These biomarkers were to draw pathological inferences on central genes or "hub genes", and genetic pathways that ultimately contribute to the phenomenon of Alzheimer's Disease.

Dataset 2 Key Terms

Differentially expressed genes (DEGs): Refer to the process used in bioinformatics and genomics to discover genes that show significant differences in expression levels between different conditions, groups, or treatments. For example, DEGs could be genes that are upregulated (increased expression) or downregulated (decreased expression) in cancerous tissue compared to normal tissue [133].

> *Disease and health both have differentially expressed genes, especially in Alzheimer's [134].*

Microarray Experiment: A microarray experiment uses technology to measure the expression levels of many genes simultaneously. There are thousands of microscopic spots in a microchip array, that are filled with a specific gene sequence that is being searched for expression levels [135].

Gene Ontology: is a comprehensive and standardized system that categorizes gene functions into biological processes, molecular functions, and cellular components. It allows researchers to annotate genes consistently, compare gene functions across species, and analyze biological data in a systematic way, leading to a deeper understanding of gene roles in various biological contexts [136].

Protein-Protein Interaction (PPI) Network: are a graphical representation of the physical and functional interactions between proteins within a cell or organism [137].

Hub genes: are genes that play central roles in biological networks, often characterized by having a large number of connections (high degree) within a network [138].

Brain-derived neurotrophic Factor (BDNF): is a protein that plays a critical role in neuronal survival, neurogenesis, synaptic plasticity, and cognitive functions. Without BDNF neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, and Huntington's disease may contribute to the progression of these conditions by impairing neuronal survival and function. It is one of the most important neurotrophins, a family of proteins that promote the survival, development, and function of neurons [139].

WW domain-containing transcription regulator protein 1 (WWTR1): is a protein domain found in many proteins involved in cellular signaling and regulation. It mediates protein-protein interactions and plays a role in cellular processes such as cell growth, apoptosis, and differentiation. It is key to keep appropriate organ size, and plays a role in cancer [140].

Braak stages: refer to a system used to describe the progression of Alzheimer's disease pathology in the brain, specifically focusing on the distribution of neurofibrillary tangles, which are abnormal aggregates of tau protein. This staging system helps in understanding the progression of the disease and correlating it with clinical symptoms and cognitive decline [141].

- Braak Stage I: Early stage with minimal cognitive impairment, often asymptomatic.
- Braak Stage II: Mild cognitive impairment may begin to be noticeable, particularly in areas related to memory.
- Braak Stage III: Progressive cognitive decline is evident, with increased memory problems and other symptoms affecting daily life.
- Braak Stage IV: More pronounced cognitive deficits, including difficulties with language, spatial orientation, and executive functions.
- Braak Stage V: Severe cognitive impairment, with significant loss of independence and function. More extensive involvement of various cognitive domains.
- Braak Stage VI: Advanced stage of Alzheimer's disease with profound cognitive impairment and severe functional decline. Patients may experience significant difficulties with basic activities of daily living.

Amyloid-beta (Aβ) 42 & β-secretase activity: is a specific form of amyloid-beta peptide that is 42 amino acids long. It is one of the most studied peptides in Alzheimer's disease research. Aβ 42 is prone to aggregation and forms amyloid plaques, which are a hallmark of Alzheimer's disease. These plaques are toxic to neurons and are associated with cognitive decline. Elevated levels of Aβ 42 in cerebrospinal fluid (CSF) are often used as a biomarker for Alzheimer's disease. Decreased levels of Aβ 42 in CSF can indicate its increased deposition in the brain, which is characteristic of the disease. β-Secretase activity leads to the production of Aβ peptides, including Aβ 42. Increased β-secretase activity results in higher levels of Aβ peptides, which can contribute to the formation of amyloid plaques in the brain [142] [143].

Data Wrangling Dataset 2

Dataset 2 was quite “dirty”, it had unnamed features, and its feature names started on index 2 of the dataset so that required some cleaning. Besides that, it did not have any missing columns or duplicate rows. This dataset had around 130 columns so it was not as big as the first Dataset.

Exploratory Data Analysis Dataset 2

Since the features of this dataset are biomarkers themselves, and they are compared against how progressed a test specimen's AD is by Braak stage, it is useful to see how the biomarker features relate to one another in a heatmap below for starters.

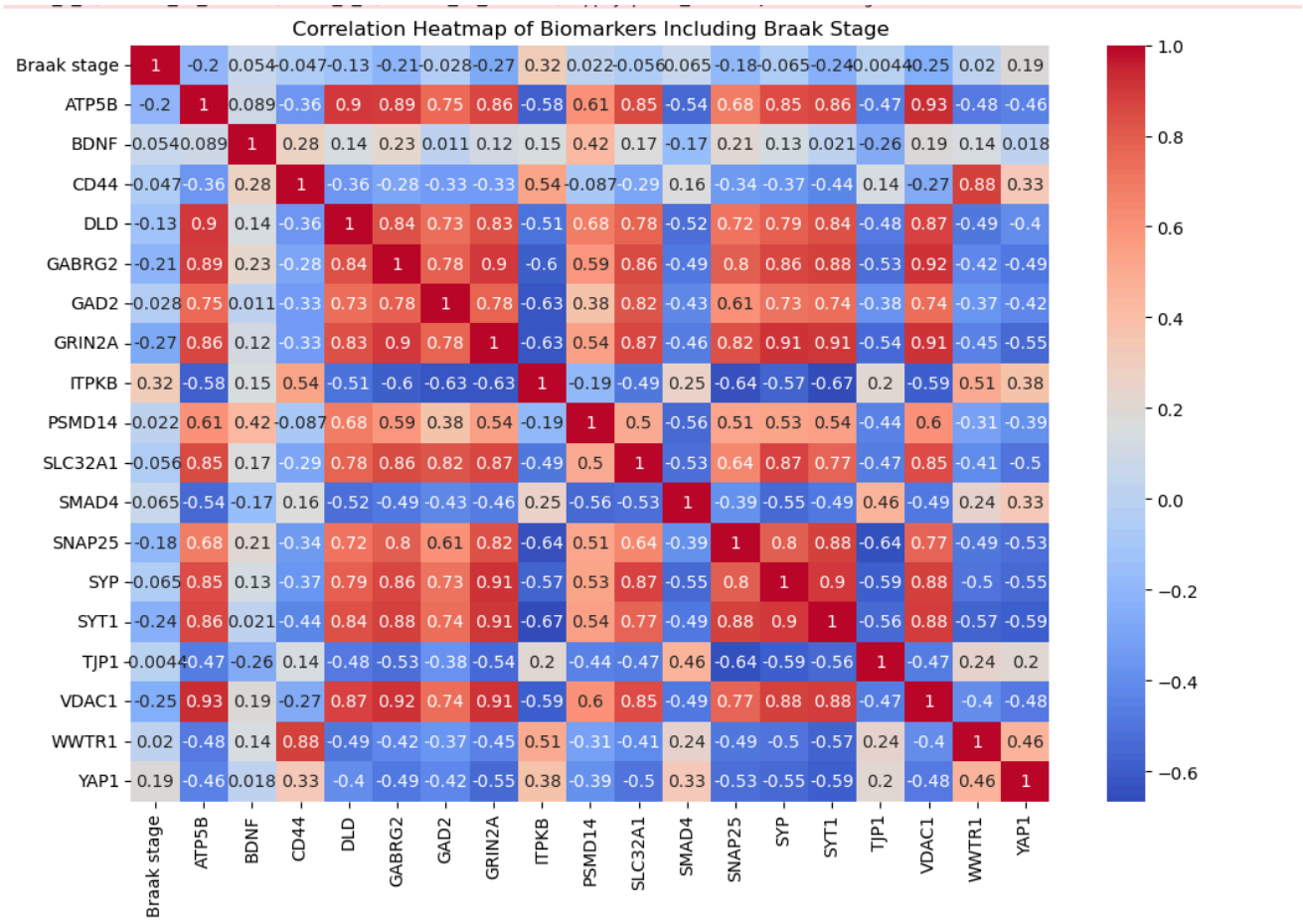

Figure. 22 shows a Heatmap that compares Biomarker correlations with themselves, along with each Braak Stage (Alzheimer's Progression). Taken from the Jupyter notebook of this paper.

Then I ran p-value permutation tests to test out a null hypothesis, that there is no difference in the Braak Stage 'V-IV' group and Braak Stage '0' group in biomarker data. As it turns out the p-value was quite high from the many permutations, so the null hypothesis is accepted that there is no difference in the Braak Stage 'V-IV' group and Braak Stage '0' group in biomarker data. The Braak Stage 'V-IV' group has markedly similar biomarker measurements than the other Braak Stage groups. This suggests that AD is markedly similar in biomarker data in an early stage and in a late stage as seen on the figure on the next page.

```
Permutation 18000:
New Stage 5 Mean: 1.0801418495502646
New Stage 0 Mean: 1.1996003478840047
Permutation Difference: -0.11945849833374012
Length of New Stage 5 Values: 378
Length of New Stage 0 Values: 1638
----------------------------------------
Permutation 19000:
New Stage 5 Mean: 1.095811567505291
New Stage 0 Mean: 1.1959842591251526
Permutation Difference: -0.10017269161986153
Length of New Stage 5 Values: 378
Length of New Stage 0 Values: 1638
----------------------------------------
Observed Difference Mean: -0.09103606686121313
Permutation Difference Mean: -0.0005984843363575084
P-value: 0.30885
```

Figure. 23 shows permutation p-value tests to determine if there is a difference between Braak Stage 'V-IV' group and Braak Stage '0' group in biomarker data. Taken from the Jupyter notebook of this paper.

Dataset 3

"Given the limitations and side effects associated with pharmaceuticals currently being used for therapy of diabetes, there is a significant need for alternative treatments. In this study, we investigated the effects of a root extract from Rhodiola rosea in a Leptin receptor knockout (db/db) mouse model of type 2 diabetes". Leptin signals to the brain to reduce hunger so in a leptin receptor knockout model some mouse groups were always hungry [148].

"Rhodiola rosea improved fasting blood glucose levels, altered the response to exogenous insulin, and decreased circulating lipopolysaccharide and hepatic C-reactive protein transcript levels. We hypothesize that these changes may in part reflect the modulation of the microbiota, resulting in improved gut barrier integrity and decreasing the translocation of inflammatory biomolecules into the bloodstream" [149].

There were two treatment groups, water-treated mice, and Rhodiola-treated mice. The CRP (gene of interest) and GAPDH (reference gene) were examined from liver extracts of the mice and were processed using DNA tests and a "PCR amplification program" [150].

Dataset 3 (columns):

- 1.) Mouse: Specimen.
- 2.) Treatment: Rhodiola vs. water.
- 3.) Sex: Gender.
- 4.) GADPH Ct Liver: Reference Gene.
- 5.) CRP Liver CT: Gene of Interest.

Conclusion

All five columns describe the information about the mouse specimen, and two columns on a reference gene, and gene of interest from the sample data. This dataset aims to view the concentrations of each reference gene and each gene of interest in order to see how each gene is expressed based on whether the mouse was in the Rhodiola group or the control water group.

Dataset 3 Key Terms

Rhodiola Rosea: Is a herb that is found at high altitudes, in cold weather. It is primarily known as an adaptogen, which are substances that help someone adapt to stress by lowering stress and cortisol. There are other therapeutic benefits to this herb such as improving biomarkers in gut microbiota within the study that is this dataset [151].

> *Rhodiola Rosea lowers inflammation within the GI Tract, thus improving gene expression in this study [152].*

CRP: C-Reactive Protien is a protein released by the liver in response to systemic inflammation. Lower levels indicate less inflammation [153].

GAPDH (Glyceraldehyde 3-phosphate dehydrogenase): An enzyme that breaks down glucose for energy, perfect to use as a reference gene[154].

Ct (Cycle threshold): Used in PCR for DNA analysis. A lower value means a higher amount of the target gene, and a higher value means a lower amount of the target gene [155].

Data Wrangling Dataset 3

There were several missing values in this dataset, and there was a column within a column which was difficult to extract and make its own feature. This dataset is quite small, and it was quite “dirty”, but it was cleaned, and the null values were handled correctly to be non-zero.

Exploratory Data Analysis Dataset 3

What we are really looking for is a lower liver ratio, a lower ratio of CRP Liver Ct/GADPH Ct Liver. A lower liver ratio means that there is less magnitude of inflammatory biomarkers for a test specimen. CRP Liver Ct measures how much liver inflammation there is. GADPH Ct Liver is a reference gene. Therefore a lower ratio to the same respective specimen is wanted. Ideally, the mice that got treated with Rhodiola Rosea should have a lower ratio but this is not seen in the statistical inferences done below. If this were the case, all of the orange dots would bunch up on the bottom of the scatter plot.

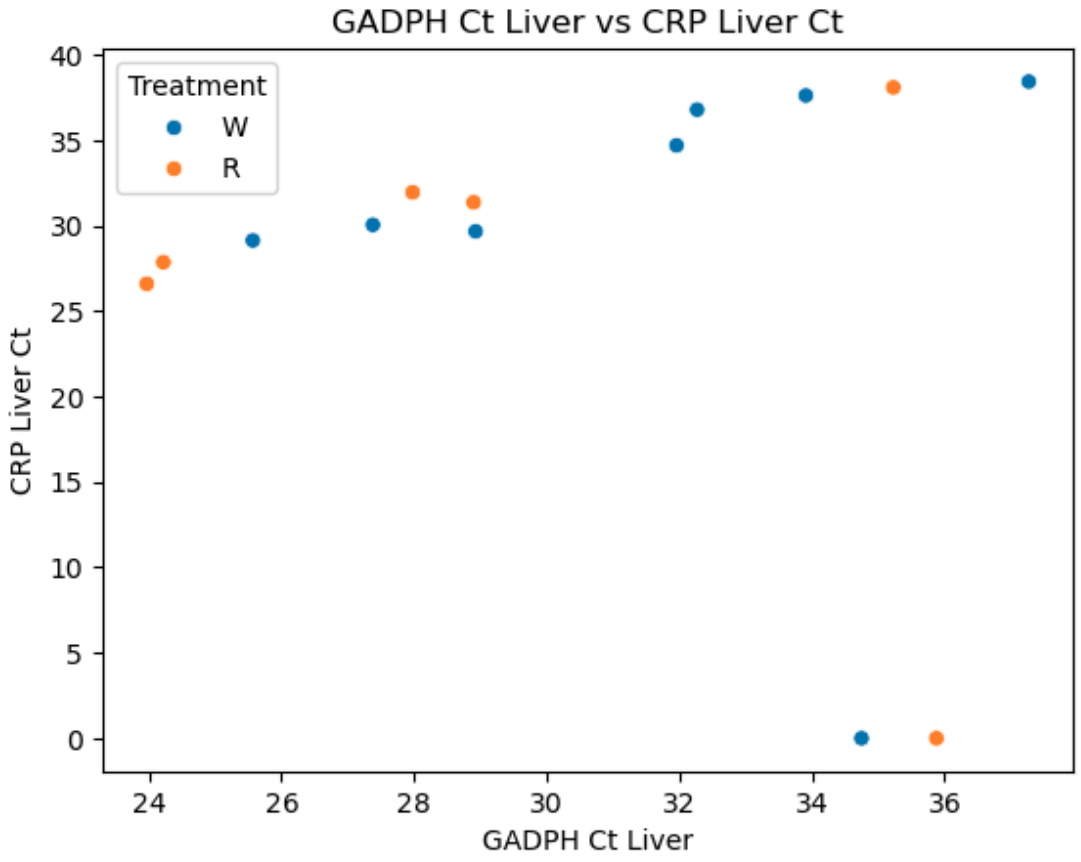

Figure. 24 shows a statistical inference of liver inflammation ratios of test specimens. Taken from the Jupyter notebook of this paper.

I also ran another p-value permutation test and with a p-value somewhat high, the null hypothesis is accepted that there is no difference in the inflammatory liver biomarkers from the Rhodiola-treated group, and the water-treated group. The Rhodiola-treated group has markedly similar biomarker measurements as the water-treated group. Statistically, the water seemed more effective than Rhodiola in showing a smaller amount of AD causing liver inflammation.

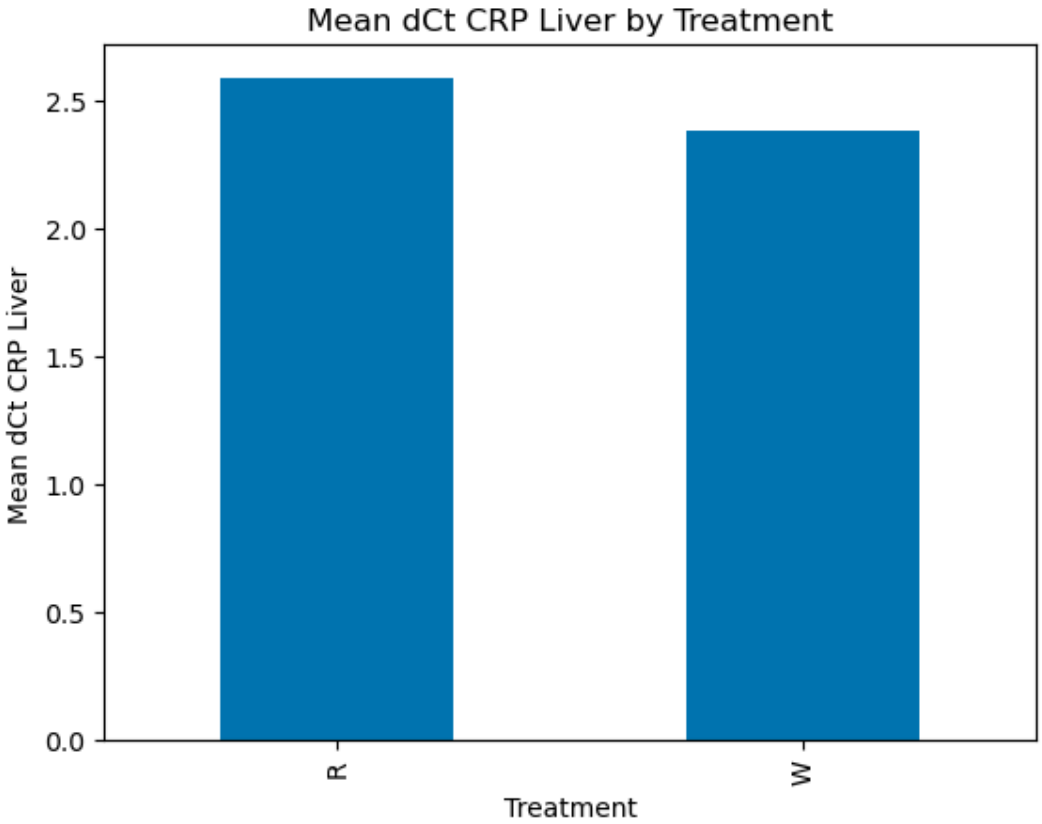

Figure. 25 shows statistically that water seems more effective than Rhodiola in reducing AD-causing liver inflammation. Taken from the Jupyter notebook of this paper.

Preprocessing and Modeling Dataset 3

This is a very small dataset, and there is not enough data to work with to make an accurately fitted model. It shows that overall the model predicts just as well as guessing. Regardless it was worth running a Logistic Regression model, to see what the results would be. The answer suggests lower inflammatory markers for the mice that got the treatment with Rhodiola Rosea suggesting, that in this logistic regression, this naturopathic herb is efficacious in lowering inflammation in the target groups that received Rhodiola Rosea which is preventative of Alzheimer's pathology. The accuracies fluctuate between 0.66 and 0.33 in different folds which seems like it predicts somewhat bad, but this is still useful to know any way to s certain degree.

```
Cross-validation accuracy scores: [0.33333333 0.66666667 0.66666667 0.33333333 0.5       ]
Mean cross-validation accuracy: 0.5
Logistic Regression Coefficients: [[-0.05081321]]
Intercept: [-0.28783944]
A lower liver ratio is associated with a higher likelihood of Treatment 'R'
```

Figure. 26 shows a run of Logistic Regression, that performs somewhat unwell because the dataset is so small. This is still worth running to examine if a lower liver ratio is more likely with the water-treated group or the Rhodiola-treated group. The results show that a lower liver ratio is more like associated with being treated with the herb Rhodiola, but the cross-validation is not good enough to make deterministic claims about this small dataset. Taken from the Jupyter notebook of this paper.

Dataset 4

Worsening colonic health is associated with age-related pathologies. In this study, it aimed to "reveal potential interactions between determinants of colonic health in aging C57BL/6J mice. Analysis of gut microbiota composition revealed an enrichment of various potential pathobionts, including Desulfovibrio spp., and a decline of the health-promoting Akkermansia spp. and Lactobacillus spp. during aging" [156]. The study also found increased gut permeability at higher ages as signs of increased inflammation and its related pathologies.

"This study demonstrates that aging is associated with pronounced changes in gut microbiota composition and colonic gene expression. Furthermore, the strong correlations between specific bacterial genera and host gene expression may imply that orchestrated interactions take place in the vicinity of the colonic wall and potentially mediate colonic health during aging" [157]. There were different gene expressions that occurred during different stages of the test specimen life, which were related to the microbiota composition at the time of life of a specimen.

Dataset 4 (columns):

- 1.) ID_REF: intensity of RNA expression on a log2 scale.
- 2.) GSM_XYZ: A mouse test group of many mice and their associated RNA expressions under them as values.

Conclusion

There are 25 mouse test groups that were split into different age spans. It was hypothesized that at older age spans, higher intestinal permeability which is linked to opportunistic microbiotia, affects colonic gene expression. Thousands of samples of colonic gene expression were measured as intra-luminal samples in RNA tests, which is the data under the mice groups [158].

Dataset 4 Key Terms

RMA Signal: A Robust Multi-array Average is a processed value that represents the intensity of RNA expression on a log2 scale in this study. This is derived from the Affymetrix GeneChip Mouse Gene 1.1 ST peg arrays used in the study to analyze the expression of various genes in the colonic mucosa and submucosa of the mice test groups [159].

RNA Transcript: An RNA transcript is the RNA strand that is produced when a gene is transcribed. Precursor mRNA (pre-RNA) is one type of RNA transcript. Pre-mRNA is processed into mature mRNA which in turn is translated into a protein. [160].

Affymetrix GeneChip Mouse: A transcript array to measure RNA transcripts from gene expressions that were used in the study of this dataset[161].

Data Wrangling Dataset 4

This is quite a large dataset there are close to 36k rows. The dataset was also quite clean with the exception of having the last row being filled will null values, so the null values were dropped by dropping the entire last row which was unhelpful.

Exploratory Data Analysis Dataset 4

It was hypothesized that at older age spans, higher intestinal permeability which is linked to opportunistic microbiota, affects colonic gene expression. Thousands of samples of colonic gene expression were measured as intra-luminal samples in RNA tests, which is the data row values under the mice groups [158].

It is interesting to see what was the highest expressed genes and the lowest expressed genes from the dataset, to try and see patterns from all of the mice test specimens. They all seemed to have the highest expressed gene, but where this dataset differs are the lowest expressed genes, suggesting that the highest expressed gene remains the same throughout the life of the test specimens and the lowest expressed genes are the genes of interest that lead to inflammatory intestinal permeability which is systemically causal of AD.

This dataset is quite simple, just looking at the genes of several test subjects and their expression rates, it does not state the age of the test specimens as a feature which makes the analysis more difficult. It can be inferred that the same genes with the lowest expressed values are more likely to be mice that are advanced in age. These mice were not treated with DSS or Rhodiola, but it is useful to see colonic gene expression.

	Highest Expressed Gene	Highest Value	Lowest Expressed Gene	Lowest Value
GSM3101204	10593865	14.093398	10340611	1.901215
GSM3101205	10593865	14.161691	10343775	1.913130
GSM3101206	10593865	14.200280	10342725	1.797553
GSM3101207	10593865	14.144030	10343254	1.916115
GSM3101208	10593865	14.090882	10341558	1.874138

Recommendations

Dangui Shaoyao San is a mixture of several herbs and is known as a multifaceted herb in Traditional Chinese Medicine. The study above proves that DSS is more efficacious in preventing Alzheimer's pathology than Rhodiola Rosea. It is recommended that supplementation with this herbal concoction over Rhodiola Rosea with the guidance of a Traditional Chinese Medicine Practitioner or Naturopathic Doctor, be given serious thought if Alzheimer's is prevalent in one's family.

Supplement companies could market DSS as an anti-neurodegenerative supplement based on the research of the studies above, and even promote a Dangui Shaoyao San supplement as anti-Alzheimer's to whatever legal limits they are able to based on the regulating bodies of their country.

Future Work

In the future, a closer look at Rhodiola Rosea can be expanded on. Rhodiola Rosea is traditionally used as an Adaptogen and an Adrenal support, and I suppose that reducing stress is good for overall health, but this work and its connections may be expanded on in further study. Not only that, but looking at definitively how age affects colonic gene expression can be expanded upon to draw more conclusions on how inflammation, and overall systemic inflammation, negatively affects colonic gene expression and AD pathways in test specimens.

MLA Works Cited

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